In the Specification:

Please amend the specification as shown:

Please delete paragraph [0007] and replace it with the following paragraph:

[0007] The amino acid sequence IKVAV **(SEQ ID NO: 1)** has been identified in other contexts as important for neuron growth and development. Self assembly of peptide-amphiphiles with the IKVAV **(SEQ ID NO: 1)** sequence have been reported. These peptide-amphiphiles may facilitate neuron growth and development in supramolecular structures formed by these peptide-amphiphiles. One feature of peptide-amphiphiles having a hydrophobic alkyl tail and the IKVAV **(SEQ ID NO: 1)** amino acid sequence in the peptide head group is that peptide-amphiphile has more than one amphiphilic moment. The peptide sequence of these and other peptide-amphiphiles can be further modified by covalent attachment of ligands or peptide sequences that can interact with various types of cells. For example, the peptide sequence Arg-Gly-Asp (RGD) occurs in fibronectin and has been found to play an important role in integrin-mediated cell adhesion. Inclusion of the RGD peptide sequence ligand into a suitable peptide-amphiphile is expected to promote cell growth and direct templated mineralization of self assembled supramolecular structures of such peptide-amphiphiles under the proper conditions. Self assembled peptide-amphiphiles are known to direct the mineralization of hydroxyapatite on the surfaces of nanofibers formed from these peptide-amphiphiles. The peptide portion of these peptide-amphiphiles can also comprise amino acid groups like cysteine, which are capable of forming disulfide bonds between adjacent peptide-amphiphiles, and also glycine which provides flexibility to the peptide portion of the molecule.

Please delete paragraph [0019] and replace it with the following paragraph:

[0019] **Figure 1** illustrates the chemical structure of a peptide-amphiphile $C_{15}H_{31}C(O)$-CCCCGGGS(P)RGD-COOH **(SEQ ID NO: 2)**.

Please delete paragraph [0021] and replace it with the following paragraph:

[0021] **Figure 3** is a schematic drawing of the chemical structures of the peptide-amphiphiles $C_{15}H_{31}C(O)$-CCCCGGGEIKVAV-COOH **(SEQ ID NO: 3)**, Molecule 1, and $C_{15}H_{31}C(O)$-CCCCGGGEIKVAV-NH$_2$ **(SEQ ID NO: 3)**, Molecule 2.

Please delete paragraph [0023] and replace it with the following paragraph:

[0023] Figure 5 is a schematic drawing of the chemical structure of the peptide-amphiphile $C_{15}H_{31}C(O)$-CCCCRFEFRFEFR-NH$_2$ **(SEQ ID NO: 4)** illustrating the important groups of the molecule as well as a representation of the magnitude and direction of two of the amphiphilic moments in the molecule.

Please delete paragraph [0025] and replace it with the following paragraph:

[0025] Although the present invention will be described in considerable detail with respect to template mediated mineralization of hydroxyapatite on self assembled peptide-amphiphile nanofibers of $C_{15}H_{31}C(O)$-CCCCGGGS(P)RGD-COOH **(SEQ ID NO: 2)**, it is not intended to be limited to this system. Other materials, minerals, biominerals, magnetic materials, conductive materials, and crystals, for example: fluoroapatite, calcium oxalate, calcite, tin hydrogen phosphate, iron oxides, iron hydroxides, and various iron oxyhydroxides, (Fe_2O_3, Fe_3O_4), TiO_2, ZnO, and versions of these materials containing substitutions of the ions, vacancies, or interstitial ions, may be nucleated and grown by the practice of this invention. The invention is not limited by the size of the crystals or crystallites formed on the self assembled peptide-amphiphiles. The formed crystals may be semi-crystalline as well. Numerous positively and negatively charged peptide amphiphile species may be used in this invention, for example $C_{15}H_{31}C(O)$-CCCCGGGS(A)RGD-COOH **(SEQ ID NO: 5)**, as well as those listed in Table 1 and Table 2. Although the present invention is described with respect to aqueous solutions, addition of other liquids or solvents like ethanol to the solution is not precluded in the practice of this invention. The invention may also be practiced by adding an effective amount of the peptide-amphiphile and salts as

powders to a surgical site, for example, where fluids containing ions needed for gelation and mineralization may be found.

Please delete paragraph [0026] and replace it with the following paragraph:

[0026] The peptide-amphiphiles and their self assembled nanofibers may promote adhesion and growth of cells on their surfaces. For example, the cell adhesion ligand RGD has been found in other contexts to play an important role in integrin-mediated cell adhesion. Peptide-amphiphile species with acidic amino acids and an amino acid with the RGD ligand could be used to mediate cell adhesion to the peptide-amphiphiles, their self assembled nanofibers, or nanofiber gels. The amino acid sequence IKVAV (SEQ ID NO: 1) has been identified in other contexts as important for neuron growth and development. Accordingly, peptide-amphiphile species with acidic amino acids and the IKVAV (SEQ ID NO: 1) sequence could be used in the practice of this invention to mediate neuron growth to the peptide-amphiphiles, their self assembled nanofibers, or nanofiber gels. The amino acid sequence YIGSR (SEQ ID NO: 6) has been identified in other contexts as important in for promoting cell-substrate adhesion among nerve cells also to play a role in axon guidance. Accordingly, peptide-amphiphile species with acidic amino acids and the YIGSR (SEQ ID NO: 6) sequence could be used in the practice of this invention to promote cell-substrate adhesion among nerve cells to the peptide-amphiphiles, their self assembled nanofibers, or their nanofiber gels. For example in dentin, the phosphophoryn protein family contains numerous repeats of the amino acid sequences Asp-Ser(P)-Ser(P) and Ser(P)-Asp. These massively phosphorylated proteins are suspected to play an important role in hydroxyapatite mineralization. Accordingly, phosphoserine residues can be incorporated into the peptide sequence which, after self assembly, allows the fiber to display a highly phosphorylated surface equivalent to that presented by a long peptide segment. This, in part, captures the repetitive organization of phosphate groups found in phosphophoryn proteins.

Please delete paragraph [0032] and replace it with the following paragraph:

[0032] Notwithstanding embodiments provided above, broader aspects of the present invention include a peptide amphiphile composition having a hydrophobic or lyophobic component and a lyophilic peptide or peptide-like component. In various preferred

embodiments, the hydrophobic component of such a composition is of sufficient length to provide amphiphilic behavior and micelle formation in water or another polar solvent system. Typically, such a component is a C_6 or greater hydrocarbon moiety, although other hydrophobic, hydrocarbon and/or alkyl components could be used as would be well-known to those skilled in the art to provide similar functional effect. Examples of such groups include but are not limited to arachidonyl, various length vinylic groups containing substituted with hydrogen or halogens such as fluorine, chlorine, bromine and iodine; acetylenic, diacetylenic and other acetylenic oligomers; various length alkene and isoprene groups substituted with hydrogen or halogens such as fluorine, chlorine, bromine and iodine. Regardless, the peptide component of such a composition can include the aforementioned RGD, IKVAV (SEQ ID NO: 1), or other sequences found especially useful for the nanofiber mineralization described herein.

Please delete paragraph [0035] and replace it with the following paragraph:

[0035] Various aspects of the present invention can be described with reference to the peptide amphiphile illustrated in Figure 1. Consistent with broader aspects of this invention, other peptide-amphiphiles, for example those listed in Table 1, may be used for the self-assembly of fibrous cylindrical micelles.

		Table 1		
<u>PA</u>	<u>N-terminus</u>	<u>Peptide (N to C)</u>		<u>C-terminus</u>
1	C16	CCCCGGGS(P)RGD **(SEQ ID NO: 2)**		COOH
2	C16	CCCCGGGS(P) **(SEQ ID NO: 7)**		COOH
3	H	CCCCGGGS(P)RGD **(SEQ ID NO: 2)**		COOH
4	C10	CCCCGGGS(P)RGD **(SEQ ID NO: 2)**		COOH
5	C6	CCCCGGGS(P)RGD **(SEQ ID NO: 2)**		COOH
6	C10	GGGS(P)RGD **(SEQ ID NO: 8)**		COOH
7	C16	GGGS(P)RGD **(SEQ ID NO: 8)**		COOH
8	C16	AAAAGGGS(P)RGD **(SEQ ID NO: 9)**		COOH
9	C10	AAAAGGGS(P)RGD **(SEQ ID NO: 9)**		COOH
10	C16	CCCCGGGS(P)KGE **(SEQ ID NO: 10)**		COOH
11	C10	AAAAGGGS(P)KGE **(SEQ ID NO: 11)**		COOH
12	C16	AAAAGGGS(P)KGE **(SEQ ID NO: 11)**		COOH
13	C22	CCCCGGGS(P)RGD **(SEQ ID NO: 2)**		COOH

14	C16	CCCCGGGSRGD (SEQ ID NO: 12)	COOH
15	C16	CCCCGGGEIKVAV (SEQ ID NO: 3)	COOH
16	C16	CCCCGGGS(P)RGDS (SEQ ID NO: 13)	COOH
17	C16	CCCCGGGSS(P)D(S(P)D (SEQ ID NO: 14)	COOH

Please delete paragraph [0036] and replace it with the following paragraph:

[0036] It should be noted that within the system examined, PAs 3 and 5 do not exhibit micelle formation, demonstrating a certain degree of hydrophobicity required for self-assembly of such compositions into the nanofibers of this invention. Depending upon desired cell or mineral growth, a phosphorylated moiety may not be required (see PAs 14 and 15). As discussed above, cellular adhesion or interaction is promoted by a particular sequence of the peptide components. With reference to PA's 10-12 and 15, a non-RGD sequence can be utilized depending upon cellular target. In particular, the IKVAV (SEQ ID NO: 1) sequence has been identified in other contexts as important for neuron growth and development. Accordingly the amphiphile compositions of this invention can include a peptide component having such a sequence for corresponding use. Lastly, with respect to Table 1, it is noted that several PA compositions do not include cysteine residues: while such a peptide sequence can be used to enhance intermolecular nanofiber stability, it is not required for micelle formation in the first instance.

Please delete paragraph [0037] and replace it with the following paragraph:

[0037] In part, the present invention also provides for a system including an aqueous solution of one or more of the amphiphile compositions described herein, and a factor or reagent sufficient to induce gelation under physiological conditions. Such gelation and/or self-assembly of various PA compositions into cylindrical micelle nanofibers can be achieved under substantially neutral pH conditions through drying, introduction of monovalent,

divalent, or higher valency ions and/or the combination of differently charged amphiphiles. The approach of using differently charged amphiphiles can also be utilized to deliver in the self assembling nanofibrous system two or more bioactive molecules, each bearing different charges and this way combining the gelation technology with the delivery of multiple biological signals. Such facile factors, as described more fully below and in several of the following examples, can extend the system and/or methodology of this invention to a variety of medical applications. These and other aspects of the present invention can be described with reference to the peptide-amphiphile, PA, compositions provided in Table 2, and with further reference to Figure 1 and Table 1.

Table 2				
PA	**N-terminus**	**Peptide (N to C)**	**C-terminus**	**Net Charge at pH7**
18	C16	CCCCGGGS(P)RGD (SEQ ID NO: 2)	COOH	-3
19	C16	AAAAGGGS(P)RGD (SEQ ID NO: 9)	COOH	-3
20	C10	AAAAGGGS(P)RGD (SEQ ID NO: 9)	COOH	-3
21	C16	CCCCGGGSRGD (SEQ ID NO: 12)	COOH	-1
22	C16	CCCCGGGEIKVAV (SEQ ID NO: 3)	COOH	-1
23	C16	CCCCGGGKIKVAV (SEQ ID NO: 15)	COOH	+1

Please delete paragraph [0046] and replace it with the following paragraph:

[0046] The portion of the peptide sequence labeled 2A in Figure 5 has cysteine amino acids capable of bonding together adjacent multidimensional peptide-amphiphiles in a self assembled nanofiber. In the molecule depicted in Figure 5, the cysteine amino acids may be

replaced with glycine amino acids to provide flexibility to the peptide portion of the molecule. The cysteine amino acids may be replaced with other polar or non-polar amino acids. Other synthetic amino acids, b-amino acids, g-amino acids with polar or non-polar substituents may be used in the practice of this invention. Incorporation monomers such as hydroxyacids are also contemplated, with the effect that the corresponding component is peptide-like in this respect. The sequence of alternating polar and non-polar moieties and more specifically the alternating polar and non-polar amino acids may be varied and the invention is not limited to disclosed combinations. The peptide may contain the amino acid sequence IKVAV **(SEQ ID NO: 1)**. Other examples of peptides with alternating hydrophobic and hydrophilic amino acids, include but are not limited to: YQYQYQ **(SEQ ID NO: 16)**; AQAQAQ **(SEQ ID NO: 17)**; YQAQYQAQ **(SEQ ID NO: 18)**; RADARADA **(SEQ ID NO: 19)**; HNHNHN **(SEQ ID NO: 20)**; HNHQHNQH **(SEQ ID NO: 21)**.

Please delete paragraph [0048] and replace it with the following paragraph:

[0048] In a preferred embodiment of the invention, a supramolecular composition is formed by mixing multi-dimensional peptide-amphiphiles containing the IKVAV **(SEQ ID NO: 1)** amino acid sequence with a monovalent cation from salts such as NaCl and KCl. Examples of suitable multi-dimensional peptide amphiphiles are Molecule 1 and Molecule 2 illustrated in Figure 3. The peptide-amphiphile has amino acids with moieties for covalent coupling. Examples of such amino acids include but are not limited to cysteine. The peptide-amphiphile also has amino acids that provide a flexible linkage within the peptide portion of the molecule. Examples of such amino acid moieties include but are not limited to gylcine.

Please delete paragraph [0052] and replace it with the following paragraph:

[0052] One advantage of the present invention is that the peptide amphiphiles self assemble to form fibers rather hollow tubes. Such fibers may be suitable for deliver or encapsulation of various cell therapies and provide close surfaces for templated tissue, bone, or nerve growth. The delivery of an effective amount of such encapsulated therapeutics to a patient may be useful in the treatment of a variety of conditions. The structure of the peptide-amphiphile may be changed to create self assembled structures having various pore sizes.

Although the present invention will be described in considerable detail with respect to self assembly of multi-dimensional peptide amphiphiles with the IKVAV **(SEQ ID NO: 1)** peptide and their use in promoting cell growth, it is not intended to be limited to this amino acid sequence or to cell growth. Other multi-dimensional peptide amphiphiles with alternating polar and non-polar amino acids sequences may self assemble and direct the growth of tissues, materials, minerals, biominerals, magnetic materials, conductive and semiconductor materials, and crystals on their surfaces. Examples of such materials include but are not limited to fluoroapatite, calcium oxalate, calcite, tin hydrogen phosphate, iron oxides, iron hydroxides, and various iron oxyhydroxides, (Fe_2O_3, Fe_3O_4), TiO_2, ZnO. Versions of these materials containing substitutions of the ions, vacancies, or interstitial ions, may also be nucleated and grown by the practice of this invention. The invention is not limited by the size of the crystals or crystallites formed on the self assembled peptide-amphiphiles. The formed crystals may be semi-crystalline as well.

Please delete paragraph [0053] and replace it with the following paragraph:

[0053] Another difference between the peptide-amphiphiles in the present invention from known amphiphilic molecules is that the present invention's peptide-amphiphiles are two-dimensional amphiphiles. The peptide-amphiphiles of the present invention have two "amphiphilic moments" oriented in different directions. One amphiphilic moment coincides with or is parallel to the backbone axis of the molecule, the second amphiphilic moment is not parallel to the backbone of the molecule and is directed across the peptide sequence of the molecule. The alkyl tail moiety of the peptide-amphiphile is much more hydrophobic than any moieties on the amino acids composing the peptide part of the peptide-amphiphile. The amphiphilic moment along the backbone of the peptide-amphiphile molecule is much stronger than the amphiphilicity across the IKVAV **(SEQ ID NO: 1)** segment. The amphiphilicity in different directions is different; it is much stronger along the backbone of the molecule than along the sides of the amphiphilic peptide segment. This molecular design may serve as a prototype for other multi-dimensional amphiphilic molecules, which may not include the peptide or alkyl moieties. In principle any molecule with two or more axes of amphiphilicity may be described as a multi-dimensional amphiphile. Multi-dimensional

amphiphiles can serve as the building blocks for supramolecular assemblies and lead to the development of new supramolecular structures that may find application in different fields of nanotechnology and biomedical applications.

Please delete paragraph [0057] and replace it with the following paragraph:

[0057] Peptide amphiphile $C_{15}H_{31}C(O)$-CCCCGGGS(P)RGD-COOH (SEQ ID NO: 2) (1) was prepared using standard solid phase chemistry; its structure is shown in Figure 1.

Please delete paragraph [0062] and replace it with the following paragraph:

[0062] In another example of this invention, the composition and method consists of using highly charged peptide amphiphile species (16carbon alkyl tail with a sequence like CCCCGGGSS(P)DS(P)D (SEQ ID NO: 14) with a -7 charge, for example) dissolved in a solution of negative ions (phosphate ions with a -3 charge), call this solution X. A second solution with a positively-charged peptide amphiphile species (such as 16 carbon alkyl tail with a sequence like ACAAGGGKRGDS (SEQ ID NO: 21) - an amine terminated PA at +1 charge) in a solution with positively-charged ions, such as Ca^{2+}; call this solution Y. In both solutions the peptide heads are charged and the structural element of the peptide can be varied, to give different charged peptide-amphiphile species, depending on the application.

Please delete paragraph [0063] and replace it with the following paragraph:

[0063] The positive and negative peptide amphiphiles alone (no added salt ions) will gel each other, reaction 1, when mixed in the right ionic ratios(1:7, (-):(+) in this instance), forming mixed peptide amphiphile nanofibers, theoretically composed of 7 positive peptide amphiphiles for every 1 of the negative peptide amphiphiles. The positive peptide amphiphile solution Y may be gelled, reaction 2, with the negative ions (for example a solution containing phosphate ion PO_4^{-3}). The negative peptide amphiphile solution X will be gelled, reaction 3, with positive ions (for example a solution containing Ca^{+2}). The positive peptide amphiphile does not gel, reaction 4, in positive ions (for example Ca^{+2}). The negative

PA does not gel, reaction 5, in negative ions (for example phosphate ion PO_4^{-3}). Mixing the positive and negative ions (calcium cation with phosphate anion) will make calcium phosphate mineral (reaction 6). When solution X is mixed with solution Y, a gel forms, reaction 7, very quickly. It is believed that mineral (calcium phosphates and possibly sodium chloride) is nucleated and grown intimately and substantially throughout the mixed-peptide amphiphile fibers. The gel formed may be the product of reactions 1, 2, 3, and 6, occurring in approximately the same time frame. The combination allows us formation of a mineralized gel at physiologic pH. This example further demonstrates that by using two distinct peptide-amphiphiles, different peptide sequences which might work well in concert with one-another (such as IKVAV **(SEQ ID NO: 1)** and YIGSR **(SEQ ID NO: 6)**) might be simultaneously combined during the assembly and mineralization process.

Please delete paragraph [0064] and replace it with the following paragraph:

[0064] This example describes the synthesis of peptide-amphiphiles with more than one amphiphilic moment, and describes the synthesis of a supramolecular composition comprised of self-assembled multi-dimensional peptide-amphiphiles. A supramolecular composition is formed by combining multi-dimensional amphiphiles containing the IKVAV **(SEQ ID NO: 1)** amino acid sequence with monovalent salts such as NaCl and KCl.

Please delete paragraph [0065] and replace it with the following paragraph:

[0065] Molecule 1, as shown in Figure 3, is a peptide-amphiphile that contains the amino acid sequence IKVAV **(SEQ ID NO: 1)** moiety with terminal end group –COOH; this sequence has been shown to promote axon outgrowth in neurons. Molecule 2, also shown in Figure 3, is a peptide-amphiphile that contains the sequence IKVAV **(SEQ ID NO: 1)** moiety with the terminal end group – NH_2, which has similarly been shown to promote axon outgrowth in neurons. The two molecules dissolve in pH 7.5-adjusted water at a concentration of about 10mg/mL. Molecule 1 has a charge of (-1) and molecule 2 has a charge of (+2) under these conditions. A self-supporting gel forms on mixing of either of the peptide-amphiphile solutions with 200 mM KCl or NaCl solutions. Examination of the gels

formed by these reactions by negative stain TEM shows that the gels are composed of nanofibers of the self assembled peptide-amphiphiles.

Please delete paragraph [0066] and replace it with the following paragraph:

[0066] In all cases self assembled gels comprised of the nanofibers were formed similar to those described elsewhere (Hartgerink et al., 2001; Hartgerink et al., 2002). In contrast no self assembly or gel formation was observed when other negatively or positively charged peptide-amphiphiles were exposed to the NaCl or the KCl at concentrations up to 6 M. The fact that molecules 1 and 2 assemble in the presence of the monovalent salts sets them apart from the other molecules studied. Experiments with negatively charged molecules that do not contain amphiphilic peptide sequences show that the charge screening by monovalent inorganic ions alone is not sufficient to induce peptide-amphiphile self-assembly. The reason for this difference may be in the structure of these molecules. Both these molecules contain IKVAV **(SEQ ID NO: 1)** sequence at the c-terminus of the peptide segment. This sequence is comprised of alternating extremely hydrophobic amino acids I and V and more hydrophilic ones such as A and K. Since the side chains of adjacent amino acids are located on opposite sides of the peptide backbone, this sequence is amphiphilic. The molecules 1 and 2 may be considered as double or two dimensional amphiphiles; one moment of amphiphilicity coinciding with the backbone axis of the molecule and amphiphilic peptide segment at c-terminus, and the second moment of amphiphilicity directed across the amphiphilic peptide segment. Previously amphiphilic peptides have been shown to assemble into ribbon like structures forming 3-D networks upon addition of monovalent salts (Zhang et al., 1995; Caplan et al., 2000). It was suggested that the function of inorganic ions in these systems is to screen charged functional groups of the peptide that facilitates supramolecular assembly of amphiphilic peptides. It is believed that a similar mechanism is involved in the self assembly of peptide-amphiphiles containing IKVAV **(SEQ ID NO: 1)** sequences in addition to the hydrophobic interactions between the alkyl parts of the molecules.